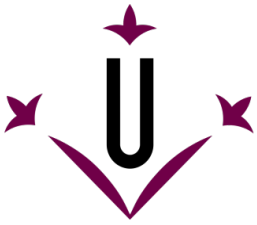

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Title: Purple, high β-glucan, hulless barley as valuable ingredient for functional food

Article Type: Research paper

Keywords: Purple barley; β-glucans; arabinoxylans; phenolic compounds; biscuits

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***Highlights (for review)**

Purple, high β-glucan, hulless barley as valuable ingredient for functional food

- Purple, high β-glucan, barley is a valuable ingredient for healthy food products
- Purple barley is high in anthocyanins and in other phenolic compounds
- 100% purple barley biscuits can easily fulfill the β-glucans EFSA health-claim
- Purple barley biscuits have acceptable physical properties
- Baking does not change dietary fiber and differentially affects phenolic compounds

Title: Purple, high β-glucan, hulless barley as valuable ingredient for functional food

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Abstract

Barley (*Hordeum vulgare* L.) stands out for its high content on bioactive compounds although it is not frequently found in human food. In this study, a purple hulless barley genotype was used to explore its food potential. β-glucans, arabinoxylans, anthocyanins and other phenolic components were determined in biscuits containing different proportions of whole barley flour and pearling fractions and compared to biscuits prepared with 100% refined (control) and 100% whole wheat flour. Barley biscuits were richer in bioactive compounds, showed higher *in-vitro* antioxidant capacity and lower estimated glycemic index with slight changes in physical properties. Baking did not affect β-glucans and arabinoxylans while it increased most of the phenolic compounds and antioxidant capacity. Barley anthocyanins were thermally unstable and exhibited high degradation rates but were partially stabilized by tartaric acid. Biscuits baked with 100% flour from purple barley grains fulfill the health claim of "high in fiber". A single biscuit provides more than 0.75g of β-glucans. Thus, one serving of four biscuits satisfies the 3g of β-glucans per day target to display the label of "reduces blood cholesterol and risk of heart disease".

Keywords: Purple barley; β-glucans; arabinoxylans; phenolic compounds; biscuits.

1. Introduction

Barley is a cereal highly variable in its morphology, development, composition and adaptation. There are two- and six-rowed barleys according to the spike structure; hulled or hulless based on whether the hulls remain adhered to the grain or not; spring or winter cultivars with different requirements for flowering; diverse grain color (black, blue, purple or yellow) due to anthocyanins or other phenolic components. These barley types differ in their physical and chemical attributes and can be processed differently for diverse commercial purposes (Baik & Ullrich, 2008).

Barley grain is rich in starch (70-80%), has an adequate protein balance (10-16%) and low lipid content (2-3%). Most barley produced in the world is used for animal feed, less than 6% for malt and a relatively negligible quantity for food. However, barley is of increasing interest as a food due to its bioactive compounds. These include β-glucans, arabinoxylans and a wide variety of phenolic compounds. β-glucans and arabinoxylans are non-starch polysaccharides and major constituents of cell walls. In barley, the β-glucan content varies between 3-11% and that of arabinoxylans from 2-9% (Izydorcyk & Dexter 2008; Hassan et al., 2017). Both are dietary fiber and their intake has been related to health benefits. The American Food and Drug Administration released a health claim associating consumption of whole grain and dry milled barley products that provide at least 3g β-glucan per day with reduced total and LDL blood cholesterol (FDA, 2008) Furthermore, the European Food Safety Authority acknowledged than barley β-glucans reduce blood cholesterol and the risk of heart disease as well as contributing to a reduction in the rise of blood glucose after meal (EFSA, 2011). In recent years, β-glucans have also received increasing attention as immune system enhancers for fighting infectious diseases, inflammatory pathologies, and some types of cancer (Hong et al., 2004; Bashir & Choi, 2017; De Graaff, Govers, Wichers & Debets, 2018). Arabinoxylans are also associated with lowering cholesterol and glucose as well as having antioxidant properties due to the presence of phenolic acids linked to their structure (Izydorczyk & Dexter, 2008; Malunga & Beta, 2015; Fadel et al., 2018)

Barley is also a good source of phenolic compounds, which can be found free or bound to fiber. Phenolic compounds are known to reduce oxidative stress associated with metabolic diseases and to promote health by modulating degenerative pathologies such as cardiovascular disease, inflammation and cancer (Calinoiu & Vodnar, 2018). Flavonoids are the main compounds in the free phenolic fraction, while phenolic acids are the most abundant among the bound one. Anthocyanins, which belong to the class of flavonoids, are normal constituents in colored barley grains.

Due to its health benefits, particularly from β-glucans, barley has been tested as an ingredient to improve the nutritional value of such wheat-based products as cookies (Sharma & Gujral, 2014) or bread (Blandino et al., 2015). However, as far as we know, the use of purple barley in the production of functional foods has not been reported. Therefore, in this study, a purple, hulless genotype was selected to explore its potential for food purposes through the following tasks: 1) determining the β-glucan, arabinoxylan and phenolic compound composition; 2) preparing biscuits containing barley flour or external barley grain fractions, analyzing their composition, and evaluating the impact of baking on the bioactive compounds compared with refined and whole wheat flour biscuits; 3) assessing their *in-vitro* antioxidant capacity, estimated Glycemic Index (eGI), and physical properties.

2. Materials and methods

2.1. Plant material

Hindukusch, an Afghan barley landrace, purple in color and with hulless grains, was used for this study (https://www.seedstor.ac.uk/search-infoaccession.php?idPlant=3707).

2.2. Flour and barley fractions

Whole grain was ground in a Foss Cyclotec 1093™ mill equipped with a 0.5-mm screen (FOSS, Barcelona, Spain). The external grain fraction was obtained by pearling until a loss of 15% of the original grain weight, using a TM-05C pearling machine (Satake, Thailand CO., LTD) at 1060 rpm.

2.3. Biscuits elaboration

Biscuits were prepared according to the AACC method 10-50.05 (AACC, 2008) with little modifications, using 112.5g flour, 32g vegetable margarine, 65g sugar, 1g salt, 1.25g sodium bicarbonate, 16.5g 6% dextrose solution and 8g distilled water. On a dry weight basis, flour represented 53% of the biscuit formulations. Flours and blends to prepare the biscuits were: **R** (100% commercial refined wheat flour), **W** (100% commercial whole wheat flour), **B** (100% whole barley flour), **30B** (70% R: 30% B), **30E** (70% R: 30% external barley grain fraction). The

ingredients were mixed in a spiral kneader for 6min. The dough was flattened to 6 mm, cut into 60mm diameter pieces, and baked in an industrial oven (PE 46 SVR, Eurofred, Italy) at 200°C for 10min. The biscuits were cooled for 30 min, stored in an airtight plastic container and kept at room temperature prior to chemical and physical analysis. They were coded according to their flour name followed by letter **b**.

2.4. β-glucan, arabinoxylan and amylose determination

β-glucan, arabinoxylan and amylose/amylopectin ratio of starches were determined using mixed-linkage β-glucan assay (K-BGLU), D-xylose assay (K-XYLOSE) and amylose/amylopectin assay (K-AMYL) kits from Megazyme (Wicklow, Ireland).

2.5. Phenolic compounds (PC) and anthocyanin analysis by UPLC-MS/MS

Free and bound PCs were extracted according to Martínez et al. (2018). Extracts were analyzed in an AcQuity Ultra-Performance TM liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS) from Waters (Milford, MA, USA). The analytical column was an AcQuity BEH C18 column (100mm × 2.1mm i.d., 1.7 µm) equipped with a Van Guard TM Pre-Column AcQuity BEH C18 (5mm × 2.1 mm, 1.7 µm), also from Waters. The mobile phase was 0.2% (v/v) acetic acid and acetonitrile for phenolic compounds (PC), and 10% acetic acid (v/v) and acetonitrile for anthocyanins. The HPLC system was coupled to a triple quadrupole detector mass spectrometer (Waters, Milford, MA, USA) equipped with a Z-spray electrospray interface for ionization, operating in negative mode $[M-H]^-$ for PCs, and positive mode $[M-H]^+$ for anthocyanins. Phenolic compounds were quantified by reference to a 0.02–25ng calibration curve of commercially available standard compounds and results expressed as µg/g dry sample. A linear response was obtained for all the available standards, as checked by linear regression analysis. Limits of detection (LOD) ranged from 0.007 to 0.09ng and limits of quantification (LOQ), from 0.02 to 0.30ng.

2.6. *In-vitro* antioxidant capacity

The Oxygen Radical Absorbance Capacity (ORAC) of the PC extracts was measured according to Huang, Ou, Hampsch-Woodill, Flanagan & Prior (2002). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as the control, with one ORAC unit being equal to the antioxidant protection given by 1µm Trolox. The antioxidant capacity was determined using a FLUOstar OPTIMA fluorescence reader (BMG Labtech), with reagents automatically transferred into a 96-well flat-bottom polystyrene microplate controlled by the OPTIMA 2.10R2 software. The reader was equipped with fluorescence filters with 485nm excitation and 520nm emission wavelengths in order to measure changes in the fluorescence of fluorescein under controlled temperature (37°C). The total antioxidant capacity was expressed as µmols of Trolox-equivalents per 100g of dry sample.

2.7. Estimated Glycemic Index

In-vitro digestibility was evaluated according to Brennan & Tudorica (2008). A non-linear model was applied to describe the kinetics of starch hydrolysis (Goñi, García-AIonso & Saura-Calixto, 1997). The area under hydrolysis curve (AUC) was calculated using the equation: *[AUC* $= C_\infty (t_f - t_0) - \left(\frac{C\infty}{k}\right) [1 - e^{-k\,(tf - t0)}]$; where C∞ corresponds to the concentration at equilibrium (t_{180}), t_f to final time (180 min), t_0 to initial time (0 min), and k to kinetic constant. The hydrolysis index (HI) was calculated from the AUC of the samples as percentage of the corresponding area of the reference white wheat bread *[HI = AUC sample/AUC wheat bread*100]*. The estimated glycemic index (eGI) was finally determined using the equation *[eGI = 0.862*HI + 8.198]* (Granfeldt, 1994).

2.8. Physical parameters

Width (W) and thickness (T) were measured according to the 10-50.05 AACC method. The texture analyzer TX-XT2 (Stable Micro Systems, Ltd. USA) with a cylindrical puncture probe (P/2) was used to measure strength. Hardness (N) was measured from the force-distance curves and calculated by the SMS Exponent Connect software. The L*, a* and b* chromatic

values were measured with a Macbeth Color-eye 3000 colorimeter (Altrincham, UK). Spectral data were obtained using an Illuminator C and a 10° observer.

2.9. Statistical analysis

All measurements were carried out in triplicate. The analysis was conducted with JMP®Pro14 (SAS institute Inc., Cary NC). Tukey-Kramer's HSD (α=0.05) was preferred for multiple comparisons.

3. Results and discussion

3.1. Bioactive compounds in flours and biscuits

The bioactive composition of the flours and biscuits prove the interest of purple hulless barley genotypes as a valuable ingredient for healthy food products. Table 1 shows the β–glucan and arabinoxylan contents of the flours and biscuits. Barley flour **(B)** was high in β-glucans, reaching 8.3g/100g sample. The β-glucan content of refined wheat flour **(R)** was only 0.2g/100g sample, which increased to 2.6g/100g sample when mixed with 30% whole barley flour **(30B).** The incorporation of 30% barley external fraction did not increase the β-glucans in refined wheat flour due to the low β-glucan content of the outer external layers of the grain. After biscuit making, the 100% barley biscuit (**Bb**) was the richest in β-glucans (4.3g/100g sample). The refined wheat biscuit **(Rb)** had 0.1g/100g sample, which increased to 1.1g/100g sample with the inclusion of 30% whole barley flour **(30Bb).** No significant differences were found between the β-glucan content of **Rb**, whole wheat biscuits **(Wb),** and biscuits containing barley external fractions **(30Eb).** As each biscuit weighed about 20g, **Bb** provides more than 0.75g of β-glucans per unit, which is the limit defined by EFSA for labelling (EFSA, 2011). Additionally, one serving of four **Bb** biscuits would satisfy the 3g of β-glucans per day goal to support the claim of reducing blood cholesterol and the risk of heart disease (EFSA, 2011).

Regarding arabinoxylans, the barley flour contained 6.3g/100g sample, being similar to whole wheat **(W)** and twice that of the control. In contrast to β-glucans, arabynoxylans are

predominant in the outer layers of the grain and **30Eb** biscuit was the richest in arabinoxylans, with similar values than those from **Wb** and four times higher than the control.

Regarding β-glucans and arabinoxylans, the major components of the barley dietary fiber, **Bb** also fulfilled the claim of "high in fiber" for products containing at least 6g fiber/100g food. **Wb, 30Bb** and **30Eb** could also be considered as "source of fiber", since they contained more than 3g fiber/100g food (Regulation EC No 1924/2006).

Hindukusch was richer in phenolic compounds (PC) than both wheat flours (Table 2). The phenolic content of refined flour was 89 µg/g sample, but when 30% of barley external fraction was added, the concentration increased to 1413 µg/g sample, similar to the 100% barley flour.

Analysis of phenolic profile of flours and biscuits was carried out by UPLC-MS/MS. Thirty-six different compounds were identified, flavanols and phenolic acids being the most abundant. Negligible amounts of free PCs were observed in the control flour. Hindukusch contained 304µg/g sample of total free PCs among which proanthocyanidins and catechins accounted for 65%, phenolic acids for 26% and flavone glycosides for 9%. Free PCs in biscuits ranged from 30µg/g sample in **Rb** to 163µg/g sample in **Bb**. Bound phenolic acids were predominant in all flours, representing 74-99% of total PC. Hindukusch contained 1030µg/g sample, twelve and three times greater that of the **R** and **W** flours respectively. Ferulic acid was the main bound component with 76-87% of the total. **Bb** and **30Eb** contained the highest amount of bound PCs (1151 and 1259µg/g sample respectively), while **Rb** had the lowest (330µg/g sample).

Anthocyanins were minor constituents compared to the phenolic acids and flavanols (Table 3). A total of 22 anthocyanins were detected in Hindukusch. These comprised pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvinidin conjugates of glucose, acetylglucose, malonylglucose, dimalonyglucose, dihexose and rutinose (Table S1). The Hindukusch anthocyanins content (37µg/g sample) agreed with earlier studies on purple barley cultivars

187 (Zhang, Jiang, Wei, & Liu, 2017; Martinez et al., 2018), but was lower than that reported by
188 Lee, Han, Kim, Back & Baik (2013), indicating a broad diversity of concentrations associated
189 with environmental and genetic factors. The most abundant anthocyanins were cyanidin
190 dimalonylglucoside (45%) and cyanidin glucoside (35%). As anthocyanins are located in the
191 pericarp and aleurone layers, flour containing the external fraction had the highest amount of
192 anthocyanins (76µg/g sample). Thus, **30Eb** biscuits showed the highest anthocyanin content
193 (13µg/g sample), followed by **Bb** and **30Bb**. We are not aware of previous data on the chemical
194 composition of foods containing purple barley flour, but the anthocyanin content detected in
195 **30Eb** was higher than that found in other functional biscuits prepared with purple wheat flour
196 (Pasqualone et al., 2015).

197 **3.2. *In-vitro* Antioxidant Capacity**

198 The ORAC values followed a similar tendency to that observed for the PCs, indicating a positive
199 correlation between the two assays (Table 2). The antioxidant capacity of refined flour,
200 22µmol Trolox/g sample, increased to 113.5µmol Trolox/g with the incorporation of 30%
201 barley external fraction. The **30Eb** biscuits showed the highest antioxidant capacity (92.6µmol
202 Trolox/g sample) exceeding that of the **Bb** (68µmol Trolox/g sample) despite having similar PC
203 content. The ORAC values observed in barley biscuits were similar to those found in some fruit
204 and vegetables measured by the same methodology (Wu et al., 2004). Although the *in-vitro*
205 antioxidant capacity may be different to the *in-vivo*, it can be estimated that cereal products
206 would provide 60-70% of their potential antioxidant capacity based on the regular
207 consumption to protect from oxidative stress disorders (Fardet, Rock & Rémésy, 2008). Thus,
208 grain varieties with high antioxidant contents for food purposes can be useful in populations
209 where fruit and vegetables are not often consumed.

210 **3.3. Association between variables**

Figure 1 shows the Principal Component Analysis of the bioactive compounds and antioxidant capacity of biscuits. The two principal components together explained 94% of the variability in the standardized data set. PCA1, explaining 78% of the variation, seems related to the phenolics and antioxidant capacity whereas PCA2, explaining 16%, was linked to dietary fiber, β-glucans being negatively correlated with arabinoxylans. **Bb** had the highest β-glucan content while **Wb** and **30Eb** were the richest in arabinoxylans. **Bb** and **30Eb** showed the highest amount in anthocyanins and PCs as well as the highest antioxidant capacity, although their phenolic profiles differed; **Bb** had more flavanols than **30Eb**, while **30Eb** was richer in free phenolic acids (Table 2). For PC and antioxidant capacity, the best choice was **30Eb**, but the best compromise was shown by the **Bb** biscuits as these were simultaneously high in β-glucans, PCs and antioxidant capacity.

3.4. Effect of the baking on the bioactive compounds and antioxidant capacity.

In order to compare the bioactive concentrations in flour and biscuits directly, the absolute values recorded in biscuits should be divided by 0.53 as this is the proportion of flour, on a dry weight basis, they contained. Figs. 2A and B show the absolute β-glucan and arabinoxylan contents of flours and the relative contents in biscuits. The values were close to those expected based on their percentage in flours, indicating that baking did not degrade β-glucans or arabinoxylans. These results were in accordance with those observed in other foods containing barley (Vashantan, Gaosong, Yeung & Li, 2002; Trogh et al., 2004; Mosele, Motilva & Ludwig, 2018).

Biscuits contained a higher PC content than expected from the percentage of flour. However, baking affected individual compounds differently. Total free PCs remained stable due to the compensation between the increase in the phenolic acids, aldehydes and flavone glycosides and the decrease of most flavanols (Fig 3 A; Table 2). Some authors have reported that thermal treatments may have a positive or negative influence on free PCs during processing. The final

result is the balance between the decarboxylation of phenolic acids by exposure to high temperatures (Wani & Kumar, 2016) and their release from the fibre. Baking also increased bound PCs, especially coumaric and ferulic acids, in all biscuits (Fig 3 A; Table 2). The most abundant phenolics in grains are bound to the fibre and can be released when exposed to thermal treatments. In addition, despite the sequential extraction of free and bound PCs from flours, some compounds may not be fully extracted and thus, the total components may be underestimated (Adom & Liu, 2002; Koddami, Wilkes & Robert, 2013).

The antioxidant capacity increased significantly after baking, as did the PCs (Fig 3 B; Table 2). This can be attributed to the above-mentioned release of phenolic acids from the food matrix, to the formation of some products from the Maillard reaction, or to the presence of other products newly formed after thermal treatment exhibiting antioxidant activity sometimes superior to that of native molecules (Chaaban et al., 2016).

Anthocyanins are thermal unstable compounds and thus, decreased after baking. Barley biscuits showed lower anthocyanin contents than their corresponding flours (44% less in **Bb**, 44% in **30Bb** and 68% in **30Eb;** Fig 4). Malvidins were the most unstable anthocyanins (89% degradation), followed by delphinidins (73%), pelargonidins (71%), petunidins (50%) and cyanidins (48%). Similar results have been reported in colored corn biscuits, and these were attributed to baking above 180°C (Žilić, Kocadağli, Vančetović & Gökmen, 2016). Thermal degradation begins with the hydrolysis of the sugar moieties, which are then degraded to chalcones. Subsequent breakdown of chalcones results in the formation of phenolic acids and carboxyaldehydes (Sui, Yi, Yap & Zhouet, 2015). It has been suggested that the degradation products retain antioxidant properties and hence, thermal degradation may not have a significant impact on the antioxidant capacity of the final product (Slavin, Lu, Kaplan & Yu, 2013).

3.5. Estimated Glycemic Index

Reducing the glycemic index is of interest in widely consumed cereal products, which are classified as moderate (55-70) to high (> 70) glycemic index foods (Atkinson, Foster-Powell & Brand-Miller, 2008). In this study, an *in-vitro* methodology was used to elucidate whether a partial replacement of refined wheat flour by purple barley flour or its fractions decreased the eGI. Table 4 shows the eGI of the biscuits and % amylose in starches. The control and **Wb** showed eGIs of 55.7 and 55.3 respectively. Substituting refined wheat with 30% barley flour or fractions reduced the eGI to 53.4 and 52.3 respectively, which ranked the products among the low glycemic index foods. The lowest eGI was 50.8 detected in the **Bb** biscuit.

The raw materials and baking process influence the eGI values; dietary fiber and high amylose starch contribute to lower glycemic index (Fardet, Leenhardt, Lioger, Scalbert & Rémésy, 2006). The eGI of barley biscuits can be attributed to their higher β-glucan and arabinoxylan contents. It is known that both components increase matrix viscosity and hinder the ability of digestive enzymes to release glucose resulting in a reduced glycemic response (Brennan & Tudorica, 2005). However, the results cannot be exclusively explained by their fiber content but also by other factors, such as the amylose/amylopectin ratio since amylose is more resistant to alpha-amylases (Sajilata, Singhal & Kulkarni, 2006). This may explain why the **Wb** had a higher eGI than **30Bb** and **30Eb** biscuits despite having a similar total fiber content.

3.6. Physical properties of biscuits and color stabilization

The physical characteristics of biscuits are shown in Table 5. The **30Bb** had similar volume and density to the control and was softer than **Wb**. The **Bb** and **30Eb** barley biscuits had a lower volume and more density than the control but similar hardness to **Wb.** Regarding color, significant differences were found in the L*, a* and b* values. The control biscuits were the lightest (L* = 76), followed by the **Wb** > **30Bb** > **Bb** > **30Eb.** This order was associated with their corresponding contents in anthocyanins and other PCs. All barley biscuits were less reddish (a*) and yellowish (b*) than wheat biscuits due to the high pH caused by the bicarbonate used

as leavening agent. In order to stabilize their purple color, the pH was lowered from 8 to 6 by adding 0.5g/100g tartaric acid to the dough and the new neutral conditions allowed lighter and redder barley biscuits without other significant physical changes (Table 5). In addition, not only was the color preserved but also a lower reduction in the anthocyanin content after baking was observed in **Bb** (19%) and **30Eb** (60%) (Fig 4). Hence, the choice of leavening agent seems to be a critical factor for the stability of anthocyanins during the baking process. More research is needed to evaluate the effect of other acidulates and their concentrations on the anthocyanin content of barley biscuit formulations.

4. Conclusions

Biscuits containing purple barley ingredients were richer in bioactive compounds, showed higher antioxidant capacity and lower eGI than refined and whole-wheat biscuits. Their physical characteristics were slightly worse than the **Rb** but similar to the **Wb**. Baking did not affect β-glucans nor arabinoxylans, favored the release of phenolic compounds from the food matrix and increased antioxidant capacity. Anthocyanins were thermally unstable and decreased after baking. The addition of tartaric acid improved their retention. Purple barley offers new interesting avenues to meet the demand for healthy products.

Declaration of Interest: none

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Figure captions

Figure 1. Principal Component Analysis of biscuits for bioactive compounds and antioxidant capacity. AN, anthocyanins; AX, arabinoxylans; βG, β-glucans; BP-acids, bound phenolic acids; F-ols, flavonols; F-ones, flavone-glycosides; FP-acids, free phenolic acids; TAC, total antioxidant capacity.

Figure 2. A) β-glucans content in flours (g /100 g sample) and biscuits (g /100 g flour in biscuits). **B)** Arabinoxylans content in flour (g /100 g sample) and biscuits (g /100 flour in biscuits). *****Relative values adjusted by the percentage of flour (dwb) contained in biscuits.

Figure 3. A) Phenolic compounds (PC) in flours (μg /g sample) and biscuits (μg /g flour in biscuit). **B)** Total antioxidant capacity (TAC) in flours (μmol Trolox /g sample) and biscuits (μmol Trolox /g flour in biscuit). *Relative values adjusted by the percentage of flour (dwb) contained in biscuits.

Figure 4. Total anthocyanins content in flours (μg /g sample) and biscuits (μg /g flour in biscuit). *Relative values adjusted by the percentage of flour (dwb) contained in biscuits.

Table 1

Table 1. β-glucans and arabinoxylans content detected in flours and biscuits.

	β-glucans	Arabinoxylans
Flours	**g /100 g flour**	
R	0.22 c	3.14 c
W	0.83 c	7.01 ab
B	8.26 a	6.26 b
30B	2.61 b	3.83 c
30E	1.10 c	8.27 a
SED	*0.31*	*0.44*
Biscuits	**g /100 g biscuit**	
Rb	0.14 c	1.51 d
Wb	0.40 c	4.15 ab
Bb	4.26 a	3.26 bc
30Bb	1.12 b	2.27 cd
30Eb	0.58 c	4.40 a
SED	*0.12*	*0.29*

Results as mean of three replicates. Values followed by different letters are significant different according to Tukey-Kramer's HSD (0.05); SED, standard error of the difference.

Table 2

Table 2. Content of Free and Bound Phenolic compounds (PC) and Antioxidant Capacity detected in flours and biscuits.

	Flours						Biscuits					
	R	**W**	**B**	**30B**	**30E**	***SED***	**Rb**	**Wb**	**Bb**	**30Bb**	**30Eb**	***SED***
Free PC			**µg /g flour**						**µg /g biscuit**			
Catechin	-	-	23.8 ^{a}	6.6 ^{c}	14.0 ^{b}	*1.18*	2.4 ^{b}	-	20.4 ^{a}	4.2 ^{b}	17.1 ^{a}	*2.42*
Catechin-glucoside	-	-	20.3 ^{a}	6.4 ^{b}	18.1 ^{a}	*1.35*	-	-	2.3 ^{a}	-	-	*0.35*
Procyanidin B3	-	-	71.6 ^{a}	20.6 ^{b}	85.0 ^{a}	*4.72*	3.7 ^{b}	2.4 ^{b}	8.8 ^{a}	-	3.6 ^{b}	*0.60*
GC-C/Prodelphinidin B4	-	-	72.0 ^{b}	23.4 ^{c}	116.6 ^{a}	*1.25*	2.6 ^{c}	3.1 ^{bc}	9.2 ^{a}	2.8 ^{c}	4.1 ^{b}	*0.31*
Other minorities	-	-	10.2 ^{b}	3.0 ^{c}	17.6 ^{a}	*0.96*	-	-	10.4 ^{a}	2.7 ^{b}	10.3 ^{a}	*0.77*
Total flavanols	-	-	197.9 ^{b}	60.0 ^{c}	251.2 ^{a}	*7.03*	8.7 ^{c}	5.5 ^{c}	51.1 ^{a}	9.7 ^{c}	35.1 ^{b}	*3.38*
p-OHBenzoic acid	nq	nq	12.6 ^{a}	11.0 ^{b}	13.2 ^{a}	*0.18*	9.8 ^{b}	9.8 ^{b}	12.0 ^{a}	10.4 ^{b}	13.4 ^{a}	*0.44*
2,4-DiOHBenzoic acid	-	nq	14.6 ^{a}	nq	16.19 ^{a}	*0.51*	-	12.2 ^{b}	19.8 ^{ab}	13.9 ^{b}	26.3 ^{a}	*2.49*
Coumaric acids	-	-	1.9 ^{b}	-	2.7 ^{a}	*0.13*	nq	1.5 ^{c}	3.1 ^{b}	3.7 ^{b}	5.8 ^{a}	*0.29*
Vanillic acid	nq	3.4 ^{c}	15.1 ^{b}	5.4 ^{c}	18.0 ^{a}	*0.77*	-	3.2 ^{b}	13.0 ^{a}	4.8 ^{b}	17.5 ^{a}	*1.46*
Ferulic acids	nq	3.7 ^{c}	3.7 ^{c}	5.2 ^{b}	6.6 ^{a}	*0.41*	10.7 ^{d}	20.3 ^{cd}	39.5 ^{b}	30.1 ^{bc}	70.0 ^{a}	*5.15*
Gallic acid	-	15.1 ^{c}	21.7 ^{a}	15.9 ^{c}	18.7 ^{b}	*0.49*	-	-	-	-	-	
Other minorities	-	7.6 ^{b}	8.2 ^{a}	6.2 ^{c}	8.1 ^{a}	*0.16*	-	8.5 ^{a}	0.8 ^{b}	nq	8.7 ^{a}	*0.49*
Total phenolic acids	-	29.7 ^{d}	77.7 ^{b}	43.8 ^{c}	83.4 ^{a}	*1.23*	20.5 ^{d}	55.4 ^{c}	88.3 ^{b}	62.9 ^{bc}	141.6 ^{a}	*9.65*
Luteolin-O-glucoside	-	-	8.6 ^{b}	7.3 ^{c}	9.6 ^{a}	*0.26*	-	-	7.4 ^{b}	6.6 ^{c}	8.3 ^{a}	*0.22*
Isoscoparins	-	-	16.9 ^{a}	14.1 ^{c}	15.3 ^{b}	*0.17*	-	-	14.2 a	6.5 ^{b}	13.5 ^{a}	*0.30*
Other minorities	0.8 ^{c}	10.4 ^{a}	2.6 ^{bc}	1.9 ^{bc}	5.1 ^{b}	*1.06*	1.1 ^{c}	4.7 ^{a}	1.6 ^{c}	0.8 ^{c}	2.6 ^{b}	*0.25*
Total flavone glycosides	0.8 ^{d}	10.4 ^{a}	28.1 ^{a}	23.3 ^{b}	30.1 ^{a}	*1.06*	1.1 ^{d}	4.7 ^{c}	23.2 ^{a}	13.9 ^{b}	24.4 ^{a}	*0.68*
Total free phenols	**0.8** ^{e}	**40.1** ^{d}	**303.8** ^{b}	**127.0** ^{c}	**364.7** ^{a}	*8.02*	**30.2** ^{c}	**65.6** ^{bc}	**162.6** ^{a}	**86.4** ^{b}	**201.1** ^{a}	*13.40*
Bound PC			**µg /g flour**						**µg /g biscuit**			
p-OHBenzoic acid	3.0 ^{c}	4.4 ^{c}	10.5 ^{b}	5.5 ^{c}	14.8 ^{a}	*0.92*	3.3 ^{b}	6.8 ^{b}	13.1 ^{a}	4.8 ^{b}	12.8 ^{a}	*1.24*
OHBenzoic acid	4.6 ^{a}	4.4 ^{a}	6.3 ^{a}	6.5 ^{a}	4.8 ^{a}	*1.42*	5.1 ^{a}	5.7 ^{a}	4.8 ^{a}	5.4 ^{a}	5.7 ^{a}	*0.52*
2,4-DiOHBenzoic acid	-	-	5.0 ^{b}	-	17.9 ^{a}	*0.33*	-	3.4 ^{b}	8.9 ^{a}	2.2 ^{b}	11.2 ^{a}	*1.17*
Coumaric acids	3.0 ^{c}	13.6 ^{bc}	130.2 ^{a}	32.9 ^{b}	149.3 ^{a}	*8.77*	16.1 ^{c}	55.3 ^{c}	136.3 ^{b}	22.6 ^{c}	218.8 ^{a}	*20.45*
Vanillic acid	1.2 ^{c}	6.2 ^{bc}	31.6 ^{a}	9.8 ^{b}	37.0 ^{a}	*2.22*	4.6 ^{b}	17.4 ^{b}	38.7 ^{a}	8.5 ^{b}	43.1 ^{a}	*4.48*
Ferulic acids	73.1 ^{c}	288.3 ^{b}	822.9 ^{a}	318.7 ^{b}	795.1 ^{a}	*50.38*	290.5 ^{b}	485.2 ^{b}	919.5 ^{a}	416.8 ^{b}	941.3 ^{a}	*73.05*
Other minorities	2.6 ^{e}	16.1 ^{c}	21.9 ^{b}	9.4 ^{d}	28.1 ^{a}	*0.52*	8.7 ^{b}	14.3 ^{b}	27.7 ^{a}	12.9 ^{b}	24.2 ^{a}	*1.94*
Total phenolic acids	87.7 ^{c}	332.9 ^{b}	1028.4 ^{a}	382.8 ^{b}	1047.0 ^{a}	*61.79*	328.3 ^{b}	588.0 ^{b}	1148.8 ^{a}	473.2 ^{b}	1257.1 ^{a}	*98.95*
Total flavone glycosides	-	-	1.8 ^{a}	-	1.7 ^{a}	*0.02*	1.6 ^{b}	0.04 ^{c}	1.7 ^{ab}	-	1.8 ^{a}	*0.06*
Total bound phenols	**87.7** ^{c}	**332.9** ^{b}	**1030.2** ^{a}	**382.8** ^{b}	**1048.7** ^{a}	*61.79*	**329.9** ^{b}	**588.0** ^{b}	**1150.5** ^{a}	**473.2** ^{b}	**1258.8** ^{a}	*98.99*
Total phenolic compounds	**88.5** ^{c}	**373.1** ^{b}	**1334.0** ^{a}	**509.8** ^{b}	**1413.4** ^{a}	*59.78*	**360.1** ^{b}	**653.6** ^{b}	**1313.1** ^{a}	**559.6** ^{b}	**1459.9** ^{a}	*95.89*
Antioxidant Capacity			**µmol Trolox /g flour**						**µmol Trolox /g biscuit**			
Total Antioxidant Capacity	**22.0** ^{c}	**44.5** ^{b}	**112.3** ^{a}	**47.9** ^{b}	**113.5** ^{a}	*2.23*	**18.1** ^{d}	**34.6** ^{c}	**68.1** ^{b}	**33.7** ^{c}	**92.6** ^{a}	*3.31*

Results as mean of three replicates. For each type of product , flours and biscuits, values within a row followed by different letters indicate significant differences according to Tukey-Kramer's HSD (0.05); SED, standard error of the difference; - not detected (< LOD); nq, not quantified (< LOQ).

Table 3

Table 3. Anthocyanins content detected in flours and biscuits.

	Pelargonidins	Cyanidins	Peonidins	Delphinidins	Petunidins	Malvidins	Total
Flours				**µg /g flour**			
B	4.48 b	30.17 b	0.040 b	2.12 b	0.142 b	0.27 a	37.21 b
30B	1.30 c	12.62 c	-	0.63 c	0.032 c	0.06 b	14.65 c
30E	7.03 a	65.43 a	0.064 a	3.27 a	0.202 a	0.22 a	76.22 a
SED	*0.223*	*1.659*	*0.003*	*0.209*	*0.014*	*0.019*	*1.673*
Biscuits				**µg /g biscuit**			
Bb	0.70 c	9.96 b	-	0.40 b	0.046 ab	0.02 bc	11.14 b
30Bb	0.26 d	4.04 c	-	0.08 c	0.001 b	-	4.38 c
30Eb	0.80 c	11.49 ab	0.044 b	0.38 b	0.035 ab	0.02 c	12.76 b
Bb + T*	1.67 a	13.39 a	0.002 b	0.88 a	0.050 ab	0.08 a	16.07 a
30Bb + T*	0.31 d	3.17 c	0.050 b	0.21 c	0.010 b	0.01 c	3.77 c
30Eb + T*	1.50 b	13.39 a	0.237 a	0.92 a	0.055 a	0.03 b	16.14 a
SED	*0.034*	*0.572*	*0.015*	*0.041*	*0.009*	*0.004*	*0.591*

Results as mean of three replicates. Values followed by different letters are significant different according to Tukey-Kramer's HSD (0.05). SED, Standard Error Difference; - not detected (< LOD); + T* biscuits with tartaric acid (0.5 g /100 g sample).

Table 4

Table 4. Starch amylose (%) and estimated Glycemic Index (eGI) for biscuits.

Amylose (%)		eGI	
Flours		**Biscuits**	
R	22.6 a	Rb	55.7 a
W	12.4 b	Wb	55.3 a
B	20.1 a	Bb	50.8 c
30B	22.1 a	30Bb	53.4 b
30E	15.3 b	30Eb	52.3 bc
SED	*0.94*		*0.49*

Results as mean of three replicates. Values followed by different letters indicate significant differences according to Tukey-Kramer's HSD (0.05). SED, Standard Error Difference.

Table 5

Table 5. Physical parameters of wheat and barley biscuits.

	Volume	Density	Hardness	Colour		
	cm^3	g/cm^3	N	L*	a*	b*
Rb	41.2 a	0.48 c	10.2 d	75.7 a	3.5 c	14.0 a
Wb	39.1 b	0.54 a	23.0 ab	70.2 b	4.1 b	11.3 b
Bb	34.8 cd	0.55 a	24.2 a	54.4 f	0.3 e	6.3 d
30Bb	41.7 a	0.45 c	18.3 c	61.4 d	0.7 e	9.2 c
30Eb	37.6 bc	0.50 bc	23.0 ab	48.5 g	0.4 e	5.8 d
Bb + T*	34.4 d	0.54 a	24.5 a	56.5 e	5.8 a	9.5 c
30Bb + T*	42.7 a	0.46 c	19.4 bc	64.8 c	2.6 d	11.9 b
30Eb + T*	37.7 bc	0.53 ab	22.9 ab	53.5 f	6.2 a	9.2 c
SED	*0.76*	*0.01*	*1.18*	*0.26*	*0.16*	*0.36*

Results as mean of three replicates. Values followed by different letters indicate significant differences according to Tukey-Kramer's HSD (0.05). SED, Standard Error Difference; + T* biscuits with tartaric acid (0.5 g /100 g sample).

Figure

Figure 1

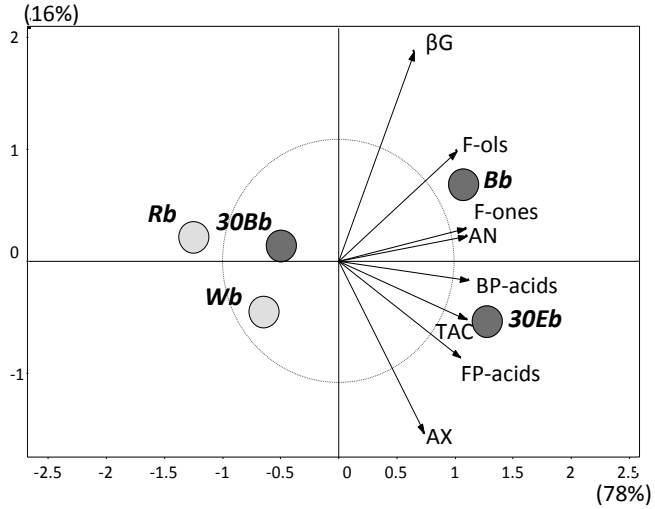


Figure 2

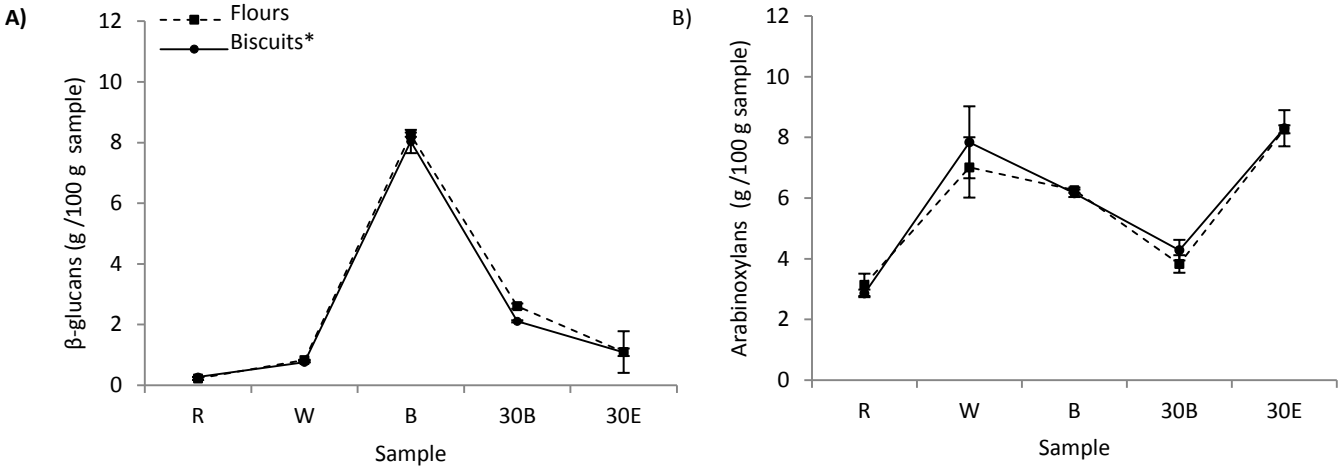

A)
Flours
Biscuits*
β-glucans (g /100 g sample)
12
10
8
6
4
2
0
R
W
B
30B
30E
Sample
B)
Arabinoxylans (g /100 g sample)
12
10
8
6
4
2
0
R
W
B
30B
30E
Sample

Figure 3

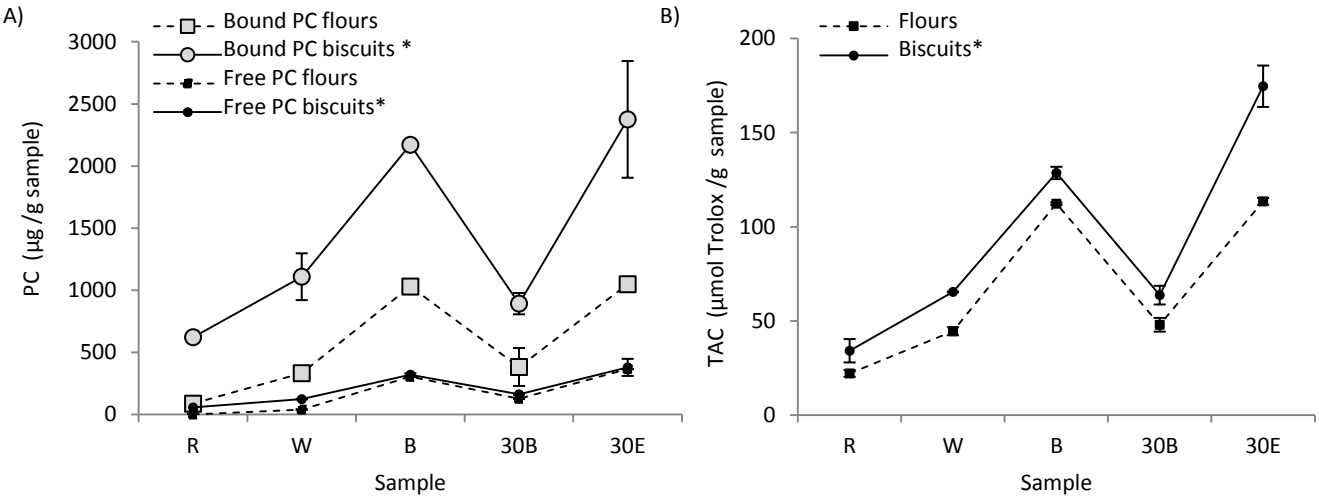
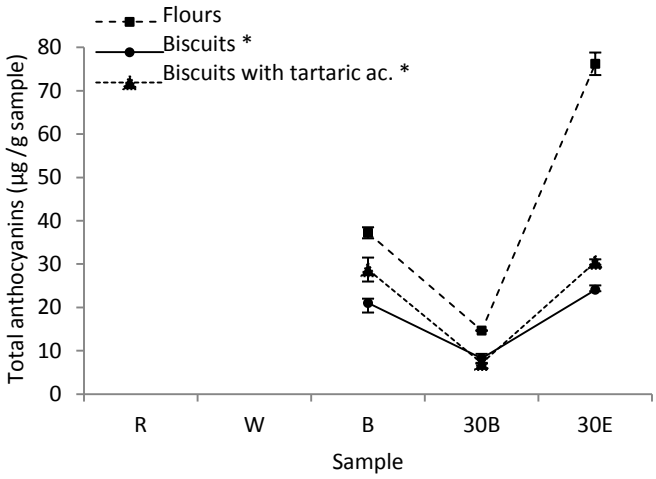

A)
Bound PC flours
Bound PC biscuits *
Free PC flours
Free PC biscuits*
PC (µg /g sample)
3000
2500
2000
1500
1000
500
0
R
W
B
30B
30E
Sample
B)
Flours
Biscuits*
TAC (µmol Trolox /g sample)
200
150
100
50
0
R
W
B
30B
30E
Sample

Figure 4


Flours
Biscuits *
Biscuits with tartaric ac. *
Total anthocyanins (µg /g sample)
80
70
60
50
40
30
20
10
0
R
W
B
30B
30E
Sample

Supplementary Material

Click here to download Supplementary Material: Supplementary Table.xlsx